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Microbial community composition and functional diversity in the rhizosphere of maize

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Abstract

This study investigates the small-scale stratification of bacterial community composition and functional diversity in the rhizosphere of maize. Maize seedlings were grown in a microcosm with a horizontal mesh (53 μM) creating a planar root mat and rhizosphere soil. An unplanted microcosm served as control. Thin slices of soil were cut at different distances from the mesh surface (0.2–5.0 mm) and analysed for bacterial community composition by PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) of 16S rDNA and tested for activities of different enzymes involved in C, N, P and S cycling. Bacterial community composition and microbial functional diversity were affected by the presence of the maize roots. The bacterial composition showed a clear gradient up to 2.2 mm from the root surface, while no such gradient was observed in the unplanted pot. Invertase and phosphatase activities were higher in the close vicinity of maize roots (0.2–0.8 mm), whereas xylanase activity was unaffected. This study shows that the changes in bacterial community composition and functional diversity induced by roots may extend several millimetres into the soil.

Introduction

In soil ecology, hierarchical approaches have gained increasing interest over the last two decades (Allen and Starr, 1982; O'Neill et al., 1986). This is because soils are considered to be extremely heterogeneous entities showing spatial variability of physical, chemical and microbiological properties at different scales (Coleman and Crossley, 1996; Parkin, 1993). This spatial heterogeneity may be explained by the patchy distribution of organic matter and/or roots in soils. For example, microscale investigations were conducted with aggregates deriving from different physical separation procedures or with different microhabitats characterized by high turnover of organic material (Beare et al., 1995; Stemmer et al., 1998). Microhabitats like the rhizosphere or the soil–litter interface have been shown to be appropriate model systems to study microbial colonisation and mechanisms driving C and N cycling (Gahoonia and Nielsen, 1991; Kandeler et al., 1999; Steer and Harris, 2000). The release of root exudates, which is influenced by plant species, plant age and root zone, creates a unique and attractive environment for microbial growth and activity (Graystone et al., 1998; Klein et al., 1988; Merbach et al., 1999). In the past, studies were mainly designed to understand the response of rhizosphere communities to different plant genotypes (Carelli et al., 2000, Di Giovanni et al., 1999; Miethling et al., 2000), mineral nutrition (Liljeroth et al., 1990; Marschner et al., 1997) or physico-chemical properties of the rhizosphere soil

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(Gelsomino et al., 1999; Gorlenko et al., 1997; Kreitz and Anderson, 1997). Due to serious methodological limitations, less is known about microscale gradients in the abundance and function of microbial communities in the rhizosphere (Asmar et al., 1995; Marschner et al., 2001). By differentiating between macerated, washed roots (rhizoplane/endorhizosphere), soil adhering to roots (rhizosphere soil) and bulk soil, Marilley and Aragno (1999) showed that some bacterial groups were specific for certain locations while others were ubiquitous. However to obtain microscale soil samples for chemical and microbiological analyses of the rhizosphere more complex experimental design and/or sampling strategies are necessary. Gahoonia and Nielsen (1991), Tarafdar and Jungk (1987) and Tarafdar and Marschner (1994) used 0.1–0.2 mm slices of soil cores that were separated from the root mat by a 53 μm nylon mesh to investigate nutrient depletion by plant roots as well as microbial, chemical and physical processes in the rhizosphere.

To study structure and function of the microbial community in the rhizosphere, various methods have been applied. Examples are direct counting of microbes (Dijkstra et al., 1987), ATP content (Youssef et al., 1989) plating on selective media (Posta et al., 1994) or the fumigation-incubation method (Helal and Sauerbeck, 1986). Since cultivation-based methods are very selective (Bakken, 1985), culture-independent methods such as fatty acid extraction (Ibekwe and Kennedy, 1998) and PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) (Muyzer and Smalla, 1998) are increasingly used for microbial community analysis in different environments. Using PCR-DGGE of 16S rDNA, Marschner et al. (2001) showed that the bacterial community composition was root zone-, plant species- and soil type-specific. While enzyme activities in the soils have been studied extensively (Kandeler et al., 1999; Nanniperi, 1994), less is known about whether and to what extent the activity of enzymes increases in the rhizosphere (Asmar et al., 1995; Badalucco et al., 1996) and how this is linked to microbial community composition. The aim of the present study was to investigate the small-scale stratification of both bacterial community composition and functional diversity in the rhizosphere of maize using molecular and biochemical techniques. Functional diversity describes a system not only by the presence or absence of a certain enzyme, but also includes the activity of an enzyme (Kandeler et al., 1996; Magurran, 1991).

Materials and methods

Experimental design

The sandy silt loam (pH 6.7; 10 m*M* $CaCl_2$) used for the experiment contained 1.3% organic C, 0.11% total N, 538 mg total P kg^{-1}, 45 mg kg^{-1} of soil-P extractable with 0.5 *M* $NaHCO_3$, 183 mg total S kg^{-1}, 161 mg organic S kg^{-1} soil, and had a CEC of 16 Ceq kg^{-1} soil at pH 7.

Maize (*Zea mays* L., cv. Jumbo) was pre-grown under non-sterile conditions in vermiculite filled in PVC tubes (length 10 cm, diameter 4.4 cm) closed at the bottom by nylon cloth impervious to roots (Figure 1 left, Gahoonia and Nielsen, 1991). Two ceramic fibre wicks were placed along the inner sides of the tubes to supply nutrient solution in accordance with the plant demand. Twelve days after germination, when root mats had developed, the nylon cloth was removed and the plants with uniformly developed root mats were transferred to a PVC tube (length 3 cm, diameter 5.6 cm) filled with soil (bulk density 1.3 g cm^{-3}). The soil column was divided into two compartments by a nylon screen with a mesh size of 53 pm into 3 cm test soil columns below and 1 cm soil layer above the screen (Figure 1, right). An unplanted microcosm served as control. The soil columns were maintained at defined moisture ($\theta = 0.21$) by placing them over small, cup-shaped sand baths each fitted with a 20 cm wick dipping into a reservoir of distilled water. Planted and unplanted microcosms were treated identically. After transplanting, new root mats developed rapidly over the nylon mesh, representing a root surface area of 24.6 cm^{-2} on the top of the nylon screen, which allowed root hairs to penetrate 0.5±0.1 mm into the test soil. Nutrient solution was supplied via the two wicks at 20 cm water tension. The ratio of nutrients in the solution was adjusted to match the demand and ratio of uptake by the maize plants using the procedure suggested by Nielsen (1984) and Asmar et al. (1995). Most (90%) of the water and nutrient uptake occurred via the two wicks from the external nutrient solution. The percentage of total N as ammonium in the nutrient solution was adjusted to maintain a near-uniform rhizosphere soil pH (Gahoonia and Nielsen, 1992b).

The experiment was conducted under controlled conditions (light intensity 280 μE s^{-1} m^{-2}, light/dark period 16/8 h, temperature 22/18 °C, relative humidity 80%). After 12 days, the soil cores of planted and unplanted treatments were frozen in liquid nitrogen and sliced with a freezing microtome to obtain rhizo-

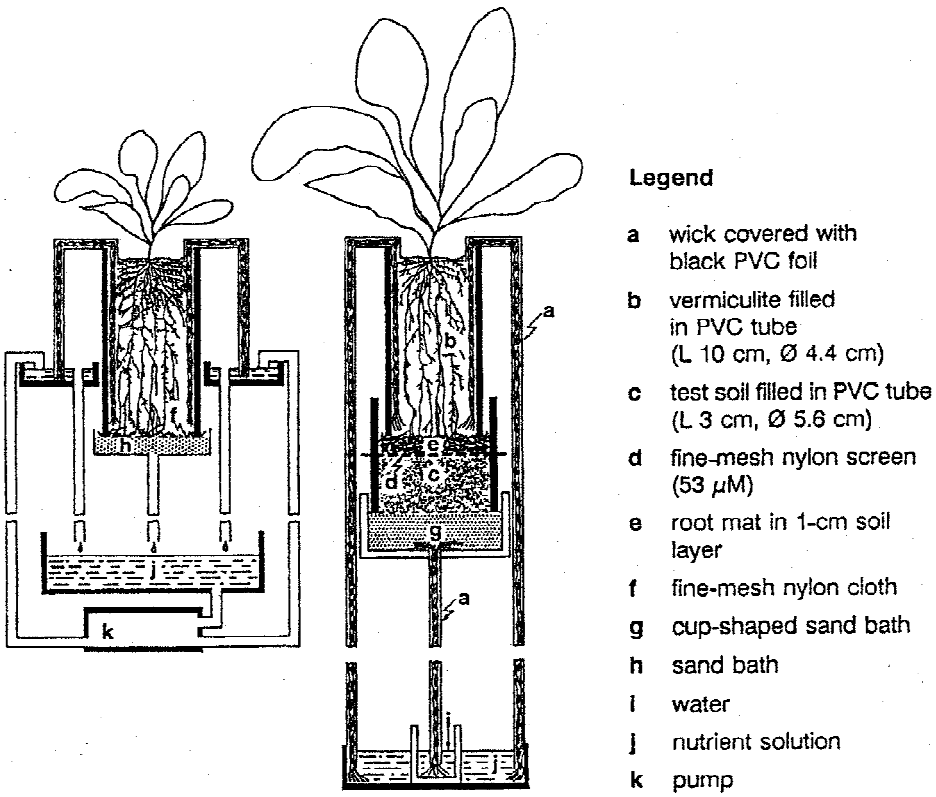

Figure 1. The pre-experimental (left) and experimental (right) set-up according to Gahoonia and Nielsen (1991).

sphere soil samples at distances of 0.2, 0.4, 0.6, 0.8, 1.0, 1.3, 1.6, 1.9, 2.2, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 mm from the mesh surface. The nylon mesh was removed just before slicing. The temperature was maintained between −10 and −20 °C during the cutting procedure. The quick freezing of the soil in liquid nitrogen does not significantly affect enzyme activities (Asmar et al., 1995). Soil samples were stored at −20 °C until analyses were performed in duplicate.

Microbial community composition

Total DNA from soil samples (approx. 300 mg) was isolated from the samples by the method of Borneman (Borneman, personal communication). Briefly, 200 m*M* phosphate buffer and 10% SDS are added to the samples which are then homogenized in a bead beater (Fast-Prep, Model FP120, BiolOl, Vista CA) at 5.5 m s^{-1} for 30 s. After proteins are removed with a protein precipitation solution (PPS®, Bio101), the DNA is bound to a silica matrix (Binding matrix®, Bio101), washed twice with an ethanol-salt solution (SEWS®, Bio101) and then desorbed into sterile water. The DNA samples were stored at −20 °C for further analysis.

For denaturating gradient gel electrophoresis (DGGE), 16S rDNA was amplified using the primer pair F984-GC and R1378 (Heuer et al., 1997). A GC-rich clamp was attached to primer F984 to prevent complete separation of the strands in the DGGE gel. For PCR, 5 μl of a tenfold dilution of the DNA extract were added to 20 μl of PCR reaction mix composed of 0.2 μl Taq polymerase (Appligene Oncor), 2 μl dNTPs (2 m*M* each) (Boeringer Mannheim), 2.5 μl 10× PCR buffer (Appligene Oncor), 0.4 μl of each primer and 14.5 μl ultrapure water. DNA was amplified in a Biometra Trio Thermocycler using 35 cycles of 1 min denaturation at 94 °C, 1 min at 54 °C for primer annealing and 2 min at 72 °C for primer extension. In the first cycle, the denaturation phase was extended to 5 min at 94 °C to prevent annealing of the primers to non-target DNA. The 35 cycles were followed by a final step of 10 min at 72 °C and cooling at 4 °C (Heuer et al., 1997). Successful amplification was verified by electrophoresis in 1.8% (wt vol^{-1}) agarose gels with SyBR green I nucleic acid stain (FMC Bio

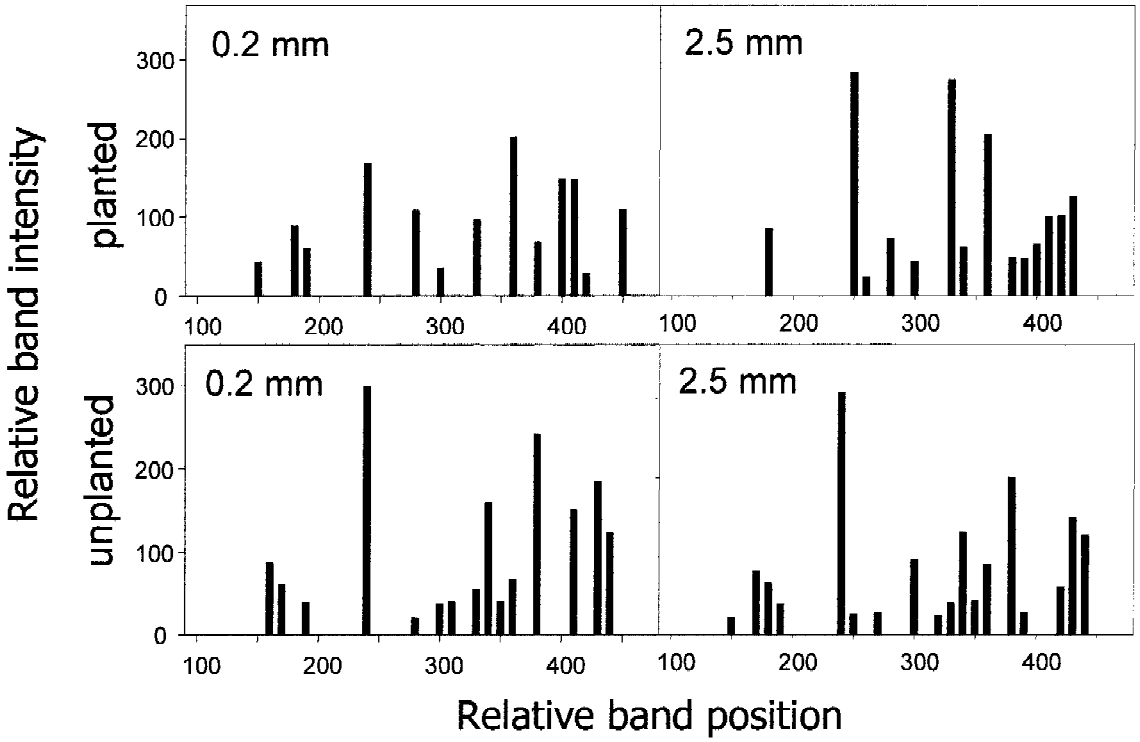

Figure 2. Digitized DGGE 16S rDNA band pattern in 0.2 and 2.5 distance from the root-soil interface and from the soil surface of the unplanted control.

Products, Rockland, USA). DGGE was performed with 8% (wt vol^{-1}) acrylamide gels containing a linear chemical gradient ranging from 35 to 55% (7 *M* urea and 40% (vol vol^{-1}) formamide). The gels were allowed to polymerize overnight. PCR products (20 μl) were electrophoresed in IX TAE buffer at 60 °C at a constant voltage of 150 V for 5 h (BIO-RAD Dcode® systems). After electrophoresis, the gels were stained for 30 mm with SyBR green I nucleic acid stain (FMC Bio Products) (10 000 foId diluted in 1× TAE) and photographed under UV light with a video imaging system. Band patterns were digitised, expressing them as band positions and band intensities of all detectable bands.

Functional diversity of the microbial community

For the determination of xylanase activity, 0.3 g of field-moist soil were incubated with 5.0 ml of a substrate solution (1.7% w/v xylan from oat spelts suspended in 2 *M* acetate buffer, pH 5.5) and 5.0 ml of 2 *M* acetate buffer for 24 h at 50 °C. Reducing sugars released during the incubation period reduced alkaline potassium hexacyanoferrate (III) and potassium hexacyanoferrate (II) and were measured colorimetrically (Schinner et al., 1996). To measure invertase activity, 0.3 g of soil were incubated with 5.0 ml of 50 m*M* sucrose solution and 5.0 ml of 2 *M* acetate buffer (pH 5.5) for 3 h at 50 °C. Reducing sugars were determined as described for xylanase activity (Schinner et al., 1996). Results of invertase activity and xylanase activity were expressed as mg glucose g^{-1} 3 h^{-1} and mg glucose g^{-1} 24 h^{-1}, respectively. Protease activity was determined as reported by Ladd and Butler (1972) with the following modifications: only 0.2 g of the soil were incubated for 2 h in 5 ml of a buffered casein solution (pH 8.1) and 5 ml of TRIS buffer (0.05*M*, pH 8.1) at 50 °C. The aromatic amino acids released were extracted with trichloroacetic acid (0.92 *M*) and measured colorimetrically using Folin-Ciocalteu reagent. The results were expressed as μg tyrosine g^{-1} $2h^{-1}$. For the determination of urease activity, 0.5–1.0 g of soil were incubated with 1.5 ml of a 79.9 m*M* urea solution for 2 h at 37 °C. Released ammonium was extracted with 13.5 ml of 2 *M* potassium chloride solution, and determined colorimetrically by a modified Berthelot reaction (Kandeler and Gerber, 1988). The method for the de-

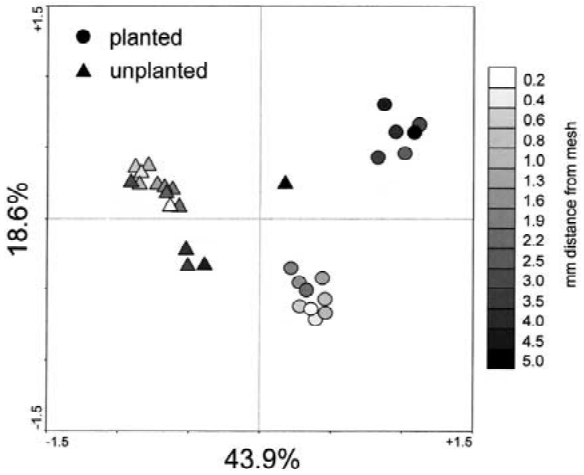

Figure 3. Ordination plot generated by principal component analysis of 16S rDNA-DGGE patterns in the rhizosphere of maize and in unplanted control. Distances from the root surface in mm.

termination of arylsulfatase was adapted to the small sample size and involves the photometrical determination of nitrophenol released by arylsulfatase using buffered potassium-p-nitrophenylsulfate as a substrate (Tabatabai and Bremner, 1970). Alkaline and acid phosphomonoesterase activities were assayed using a modified disodium phenylphosphate method: 0.3 g of soil were incubated in 2.0 ml of 0.2 *M* borate buffer (pH 10.0) for alkaline phosphatase and 2.0 ml of acetate buffer (pH 5.0) for acid phosphatase and I ml buffered phenylphosphate solution at 37 °C for 3 h; released phenol was photometrically determined (Schinner et al., 1996). The results of the enzyme measurements were expressed on the basis of oven dry (105 °C) soil.

Statistics

Community compositions based on band intensity and position of 16S rDNA DGGE patterns and enzyme activities were compared by performing a principal component analysis (CANOCO 4.0, Microcomputer Power, Ithaca, USA). Community similarities were graphed by using ordination plots with scaling focused on intersample differences (Jongman et al., 1995).

Non-linear regression analyses were applied to describe the vertical distribution of enzyme activity within the rhizosphere. The activity data were best fitted by an inverse function of the type $y = a + (bx^{-1})$. Piecewise linear regression, based on $y = b_{01} + b_1x_1 + b_{02} + b_2x_2$, was used to estimate the dimension (corresponding with the breakpoints of the model) of the rhizosphere. The quasi-Newton estimation method and the least squares as loss function were applied to estimate the parameters of the piecewise linear regression model. Based on the results of piecewise linear regression analysis the data were pooled into the inner rhizosphere (0.2–1.0 mm) and outer rhizosphere (1.3–5.0 mm). Differences between planted and the unplanted microcosms were compared by an independent *t*-test.

The band patterns of DGGE were compared statistically by principal component analysis (PCA) (Marschner et al., 2001).

Results

Microbial community composition

The composition of the bacterial community in the rhizosphere was determined by image analyses of 16S rDNA band profiles using both band position and band intensity as parameters. A total of 29 different bands were detected, of which 75% occurred in almost all samples. Nevertheless, some distinct differences could be observed. An example of the digitized band pattern at 0.2 and 2.5 mm from the mesh surface of the planted and unplanted microcosm is shown in Figure 2. In the planted microcosm, the band patterns of the two distances differ considerably, whereas they are very similar in the unplanted control. Principal component analysis (PCA) of the banding patterns revealed that the bacterial communities of the planted microcosm differed from those of the unplanted control (Figure 3). The two treatments are separated along the first principal component which explained 43% of the total variance.The microscale variability of the bacterial community within the rhizosphere is evident along PC2 (18.6% of the total variance). In the planted microcosm the community composition in the first 2.2 mm from the mesh surface is clearly different from those of the samples further away from the mesh. A gradient in community composition exists within the first 2.2 mm from the root surface. In contrast, the communities in the unplanted controls were unaffected by the distance from the mesh.

Functional diversity of the microbial community

The presence of roots also induced small-scale variability of xylanase, invertase, alkaline and acid phosphatase activities in the soil (Figures 4 and 5). With increasing distance from the root surface the gradient

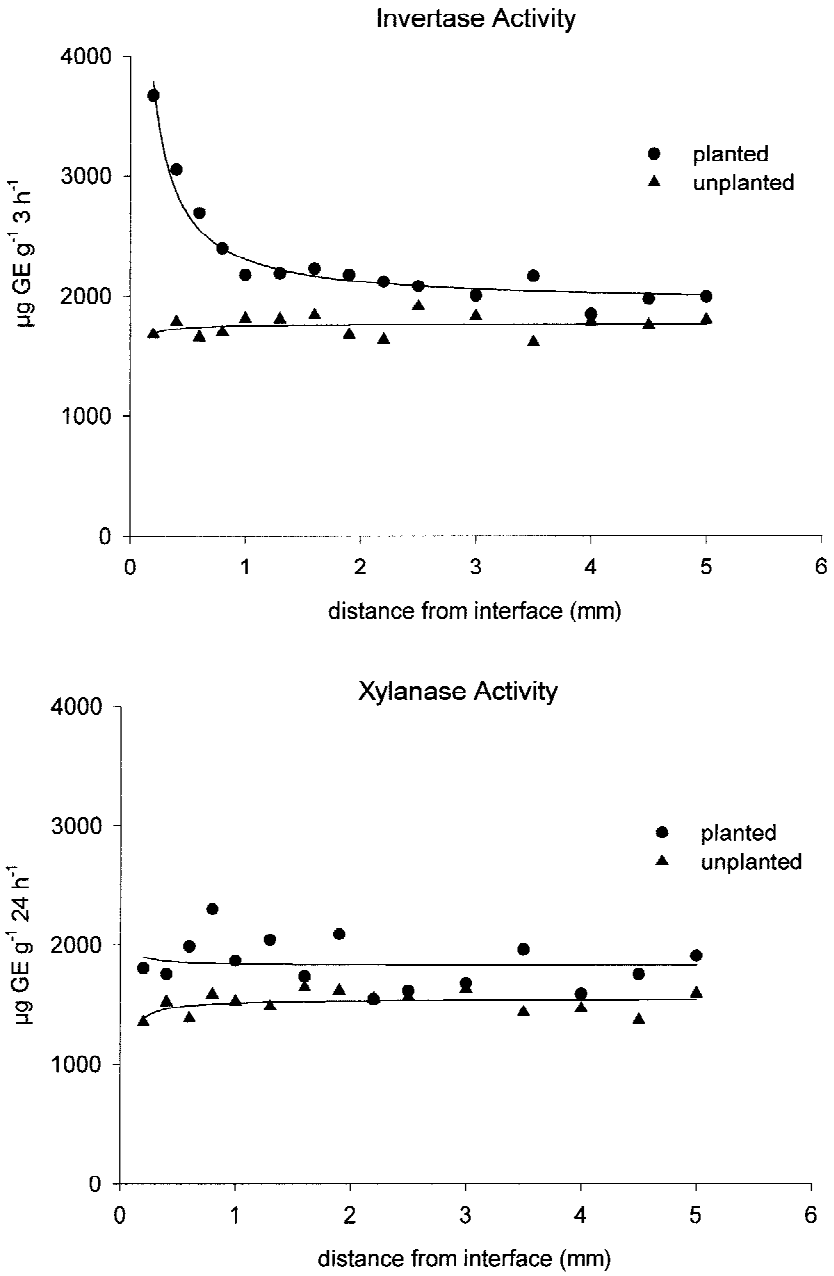

Figure 4. Small-scale variability of xylanase and invertase activities in the rhizosphere of maize and in the unplanted controls.

of invertase, alkaline and acid phosphatases decreased following the inverse function ($y = b_0 + b_1 x^{-1}$; $P < 0.001$); this function could not be applied for xylanase activity. Results of the non-linear regression analyses for measured enzyme activities are given in Table 1. Similar to invertase, alkaline and acid phosphatase, the activities of protease, urease and arylsulfatase also decreased with increasing distance from the root surface, and the distributions were described by the inverse model. As expected, enzyme activities showed no gradient in the unplanted controls (Figures 4 and 5, Table 1). Piecewise linear regression indicates that the dimension of the rhizosphere effect depends on the enzyme investigated (Table 1). A rhizosphere effect was detected up to 0.85–0.90 mm for invertase and acid phosphatase activity (breakpoint of piecewise lin-

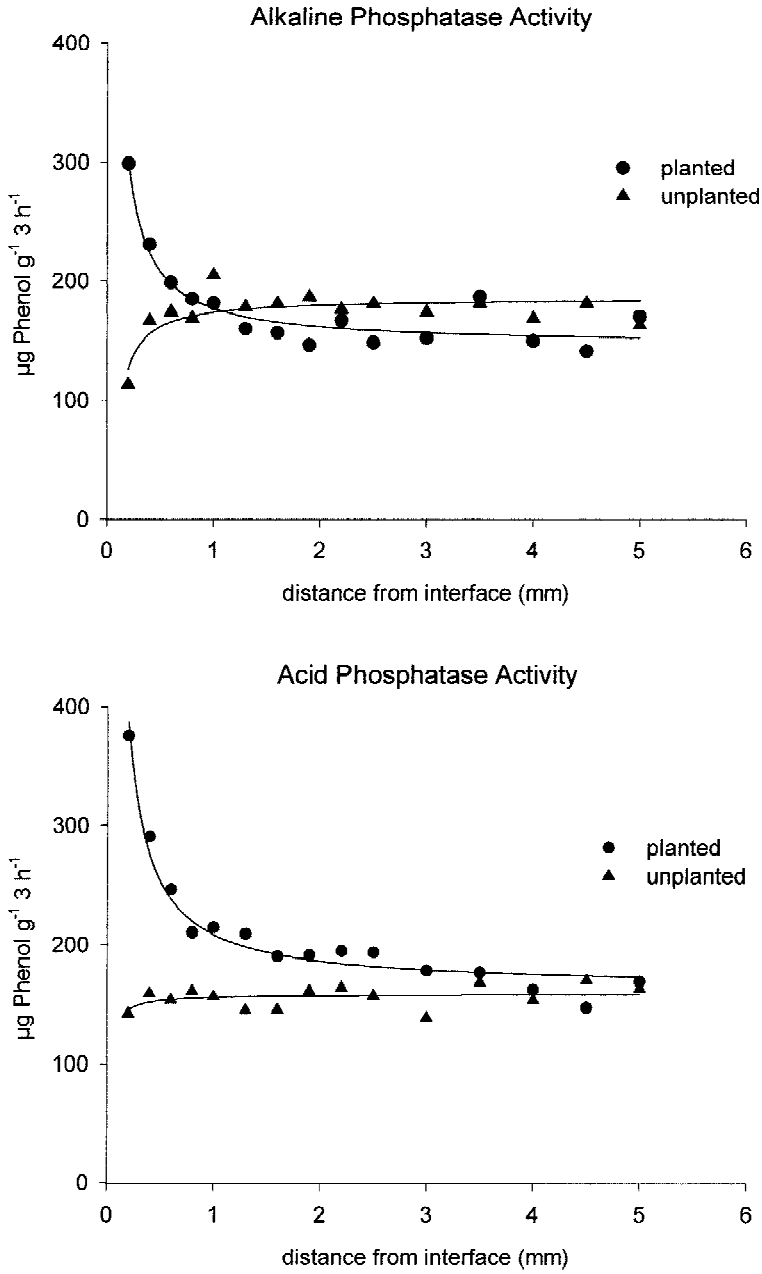

Figure 5. Small-scale variability of alkaline and acid phosphatase activities in the rhizosphere of maize and in the unplanted controls.

ear regression) and in the range of 4.39–8.72 mm for alkaline phophatase, urease, protease and arylsulfatase activities. For xylanase activity a theoretical scale of the rhizosphere effect of 33.8 mm was calculated (Table 1). Xylanase, invertase, acid phosphatase and protease activities in the planted pot differed from the unplanted pots over the entire distance from the root surface investigated in the first 1.0 mm from the mesh surface and at a distance of 1.3–5.0 mm (Table 2).

Multivariate statistics were used to assess the functional diversity of the soil microbial community according to Kandeler et al. (1996). In the principal component analysis of the data of all seven enzyme activities, PC1 and PC2 explained 57.5% and 34.7% of the total variance, respectively (Figure 6). Length and

Table 1. Results of non-linear regression analyses ($y = b_0 + b_1 \cdot x^{-1}$) to express the variation of soil enzyme activity with increasing distance from the surface of maize roots and unplanted soils. The scale (x) of the rhizosphere is based on the results (breakpoints) of piece wise linear regression ($y = b_{01} + b_1 \cdot x_1 + b_{02} \cdot x_2$). Given are the equation constants (b_0), equation coefficients (b), R^2, level of significance (NS no significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) and scale of rhizosphere (x of breakpoints, mm)

	Parameters of inverse function: $y = b_0 + b_1 \cdot x^{-1}$							
	Planted				Unplanted			
	b_0	b_1	R^2	x [mm] of breakpoint	b_0	b_1	R^2	x [mm] of breakpoint
invertase	1933.9	370.2	0.96***	0.9	1770.0	−17.0	0.06	−9.3
xylanase	1825.0	15.1	0.01	n.d.	1548.2	−33.8	0.20	−34.5
urease	43.6	9.5	0.41**	7.1	67.8	−4.7	0.41*	37.6
alkaline phosphatase	146.4	30.8	0.91***	4.4	185.8	−12.0	0.61**	6.4
acid phosphatase	163.9	33.6	0.96***	0.9	158.7	−2.8	0.14	−8.3
protease	379.2	35.4	0.33*	7.2	206.9	−3.5	0.14	−11.6
arylsulfatase	8.9	1.1	0.39*	8.7	10.4	−0.3	0.07	−2.3

n.d.: not determined, since no break point could be calculated within the first 5 mm.

Table 2. Enzyme activities of planted and unplanted soils close to (0.2–1.0 mm) and far to (1.3–5.0 mm) the root mat. Given are the means (± standard deviation) of the sampling points within the near (0.2–1.0 mm, $n = 5$) and far rhizosphere (1.3 – 5.0 mm, $n = 10$), explained variation (R^2) and level of significance (P). NS no significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

	t-Test					
	0.2–1.0 mm			1.3–5.0 mm		
	Planted	Unplanted	P	Planted	Unplanted	P
invertase	2795.9±588.0	1724.9±67.6	*	2076.1±120.5	1764.6±96.0	***
xylanase	1942.3±217.5	1473.8±99.9	**	1789.7±195.4	1533.0±91.7	**
urease	62.1±23.0	57.4±8.6	n.s.	49.0±12.8	65.7±8.8	**
alkaline phosphatase	219.9±48.6	165.6±3.2	n.s.	157.8±13.7	177.3±7.1	**
acid phosphatase	267.5±68.5	154.5±7.4	*	181.1±18.3	156.6±10.6	**
protease	459.2±67.2	199.8±10.0	**	394.0±76.5	205.0±12.8	***
arylsulfatase	11.0±2.8	9.8±1.2	n.s.	9.5±2.0	10.2±1.5	n.s.

direction of the arrows indicate that protease, alkaline phophatase and urease activities contributed considerably to PC1 which separated the planted and unplanted microcosms. The microscale variability of the functional diversity as a function of the distance from the mesh is evident along PC2. In the planted microcosm, the functional diversity in the first 0.8 mm from the mesh surface differed from the samples further away. No such a gradient was observed in the unplanted microcosm. Higher invertase and acid phosphatase activities and lower xylanase activity in the close vicinity (0.2–0.8 mm) of the roots were responsible for these results (Figure 6).

Discussion

Microbial community composition

This study showed a rhizosphere effect on both bacterial community composition and microbial functional diversity. A similar rhizosphere effect was already observed in earlier studies on bacterial population density (Foster, 1986) and composition (Marilley and Aragno, l999), DNA content (Macrae et al., 2001), alkaline phosphatase activity (Tarafdar and Jungk ,1987), phospholipid fatty acid (PLFA) profiles (Steer and Harris, 2000), pH (Gahoonia and Nielsen, 1992b) and for nutrient concentration (Gahoonia and Nielsen, 1991). The use of a mesh impervious to roots, in combination with thin slicing of the soil adjacent to

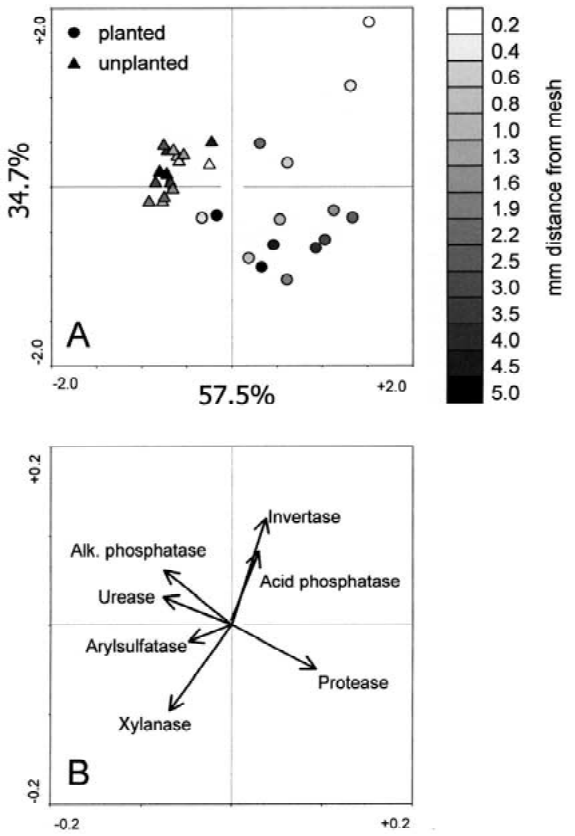

Figure 6. Ordination plot of principal component analysis of enzyme activities of samples (A) and enzymes (B) in the rhizosphere of maize and in inplanted controls. Distances from the root surface in mm.

the mesh, allows an exact determination of the extent of the rhizosphere.

The bacterial community composition in the planted microcosm differed significantly from that of the unplanted control even up to 5 mm away from the mesh (Figure 2). Since about 75% of a total of 29 different bands yielded by the DGGE were found in both planted and unplanted treatments, most soil bacteria appear to be ubiquitous (Borneman and Triplett, 1997; Gelsomino et al., 1999; Marschner et al., 2001). In the study by Marilley and Aragno (1999) members of the *Cytophaga-Flexibacter-Bacteriodes* phylum were ubiquitous, being detected in the soil, rhizosphere and rhizoplane. Rhizosphere-specific bacteria on the other hand may respond chemotactically to components of root exudates such as sugars, organic acids, aromatic compounds and amino acids (De Troch and Vanderleyden, 1996). The migration of bacteria in soil towards growing seedlings or toward the attractants glycine and aspartic acid was shown by Bashan and Holguin (1997). In addition, bacteria may be translocated passively via mass flow induced by small gradients of moisture content in the rhizosphere caused by plant transpiration. About 10% of the bands in DGGE occurred at high intensity only in the rhizosphere and, therefore, represent rhizosphere-specific bacteria. In other studies, rhizosphere-specific bacteria have been identified as *Pseudomonas* sp. (Marilley and Aragno, 1999) or Gram-positive (low G+C) bacteria (Macrae et al., 2001). More investigations are needed to clarify the phylogenetic identity of these species or groups of species. The bacterial community composition was changed by the presence of roots within 12 days, therefore these species are probably r-strategists that are able to proliferate rapidly in response to an abundance of substrate such as root exudates (Killham, 1994). PC analyses yielded two separate groups within the rhizosphere, one extending up to 2.2 mm from the mesh and the other from 2.5 to 5.0 mm. This indicates that the conditions in the rhizosphere in immediate vicinity of the root (up to 2.2 mm) differ from those in greater distance from the root (2.5–5.0 mm). In another study using a similar experimental system (Macrae et al., 2001), the rhizosphere, characterised by a higher DNA content, extended less than 1 mm from the rhizoplane of canola (*Brassica napus*). The microscale variability is likely to be due to changes in substrate amount and composition within the rhizosphere.

The root effect on the community composition beyond the zone of stimulated activity in the present study could be due to easily diffusible, root-borne signalling substances such as flavonoids that play an important role in host-rhizobium recognition (Stacey et al., 1995). It is interesting to note that the community composition in the planted microcosm showed a gradient with increasing distance from the mesh surface, while no such gradient was observed in the unplanted control. This supports the idea of a root-borne, easily diffusible, signalling substance. The distance over which these compounds penetrate soil and influence the microbial community structure is also likely to depend on the physical characteristics of the core, such as its structure, compaction and water potential and the initial distribution and activity of the microbial biomass (Gaillard et al., 1999).

Functional diversity of the microbial community

Univariate statistical analysis revealed that the activities of most soil enzymes studied were affected by the

presence of the maize roots (Table 1). The exception was arylsulfatase, which showed no significant differences between planted and unplanted treatments. The extent of the rhizosphere varied between the different enzymes: Invertase and acid phosphatase activities decreased within the first 0.9 mm by about 50%, whereas alkaline phophatase, urease and protease were affected in more than 4 mm distance from the roots. The differences between planted and unplanted microcosms as well as the changes in enzyme activity within the rhizosphere can be explained by differences both in microbial community composition (Figure 2) and substrate availability. While the microbial community composition will determine the potential for enzyme synthesis, the release of root exudates will modify the actual rate of enzyme production and the fate of produced enzymes. Given the high invertase activity close to the roots, maize roots apparently released substaintial amounts of sucrose. Acid phosphatases are not produced by bacteria. The high activity of this enzyme in the first millimetre of the rhizosphere is therefore due to release by fungi or roots (Asmar et al., 1995). The contribution of roots as a source of other enzymes is not known. The importance of the close vicinity of the root–soil interface for organic matter transformation was also shown in previous measurements of the C/N ratio at a distance of 0.2–0.6 mm from the root mat (Bek et al., 1994; Gahoonia and Nielsen, 1992a). This ratio in the accumulated organic matter ($\Delta C/\Delta N$) at distances of 0.2, 0.4 and 0.6 mm was 6.7, 6.6 and 6.4, respectively, which corresponds to C/N ratio of the microbial biomass. In contrast, the ratio in the unplanted soil was 9.6 (Gahoonia and Nielsen, 1992b). Further investigations should clarify whether a higher turnover of the microbial biomass in the close vicinity (0.2–0.8 mm) of maize roots could have contributed to the higher enzyme release from dead cells in this zone. Xylanase, invertase, acid phosphatase and protease remained at a higher level than in the unplanted control even in a distance of 1.3–5 mm (Table 2). This may be explained by the differences in bacterial community composition between the planted and the unplanted microcosm.

Higher activity of several enzymes can be interpreted as a greater functional diversity of the microbial community (Kandeler et al., 1996; Magurran, 1991) in the rhizosphere of maize. PCA analyses demonstrated clearly that increased acid phosphatase and invertase activities were responsible for the higher functional diversity in the close vicinity of the roots, whereas differences in protease and alkaline phosphatase activities mainly caused the separation of planted and unplanted microcosms (Figure 6).

This study showed that plant roots have a major impact on the composition and function of the microbial community. Changes in community composition are reflected in changes in the activity of most enzymes studied. Further research is required to clarify the interdependence between microbial community composition and function. In conclusion, the characterization of the composition and function of the rhizosphere communities can help to understand the role of micro-organisms in plant nutrition, growth promotion and disease interactions.

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